



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/415	A1	(11) International Publication Number: WO 99/17770 (43) International Publication Date: 15 April 1999 (15.04.99)
<p>(21) International Application Number: PCT/US98/21259</p> <p>(22) International Filing Date: 6 October 1998 (06.10.98)</p> <p>(30) Priority Data: 60/061,142 6 October 1997 (06.10.97) US</p> <p>(71) Applicant (for all designated States except US): BASF AKTIENGESSELLSCHAFT [DE/DE]; D-67056 Ludwigshafen (DE).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): ARNOLD, Lee, D. [CA/US]; 216 Ruggles Street, Westborough, MA 01581 (US). XU, Yajun [CN/US]; 26 Smith Street, Westborough, MA 02181 (US). BARLOZZARI, Teresa [IT/US]; 24 South Woodside Avenue, Wellesley, MA 02181 (US). RAFFERTY, Paul [GB/GB]; Knoll Pharmaceuticals, R5 Pennyfoot Street, Nottingham NGF 1GF (GB). HOCKLEY, Michael [GB/GB]; Knoll Pharmaceuticals, R5 Pennyfoot Street, Nottingham NGF 1GF (GB). TURNER, Allyson [GB/GB]; Knoll Pharmaceuticals, R5 Pennyfoot Street, Nottingham NGF 1GF (GB).</p>	<p>(74) Agents: WAGNER, Richard, W. et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02421 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: INDENO[1,2-C]PYRAZOLE DERIVATIVES FOR INHIBITING TYROSINE KINASE ACTIVITY</p> <p>(57) Abstract</p> <p>Chemical compounds that are derivatives of indeno[1,2-c]pyrazole are inhibitors of tyrosine kinase activity. Several of the tyrosine kinases, whose activity is inhibited by these chemical compounds, are involved in angiogenic processes. Thus, these chemical compounds can ameliorate disease states where angiogenesis or endothelial cell proliferation is a factor. Compounds of this invention also inhibit the induction of vascular hyperpermeability and the associated formation of edema, ascites, and exudates.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INDENO[1,2-C]PYRAZOLE DERIVATIVES FOR INHIBITING TYROSINE KINASE ACTIVITY

BACKGROUND OF THE INVENTION

Protein tyrosine kinases (PTKs) comprise a large and diverse class of
5 proteins having enzymatic activity. The PTKs play an important role in the control
of cell growth and differentiation (for review, *see* Schlessinger & Ullrich, 1992,
Neuron 9:383-391).

For example, receptor tyrosine kinase mediated signal transduction is
initiated by extracellular interaction with a specific growth factor (ligand), typically
10 followed by receptor dimerization, transient stimulation of the intrinsic protein
tyrosine kinase activity and phosphorylation. Binding sites are thereby created for
intracellular signal transduction molecules and lead to the formation of complexes
with a spectrum of cytoplasmic signalling molecules that facilitate the appropriate
cellular response. (*e.g.*, cell division, metabolic effects, responses to the
15 extracellular microenvironment) *see* Schlessinger and Ullrich, 1992, *Neuron* 9:1-20.

With respect to receptor tyrosine kinases, it has been shown also that tyrosine
phosphorylation sites function as high-affinity binding sites for SH2 (*src* homology)
domains of signaling molecules (Fantl *et al.*, 1992, *Cell* 69:413-423; Songyang *et*
al., 1994, *Mol. Cell. Biol.* 14:2777-2785; Songyang *et al.*, 1993, *Cell* 72:767-778;
20 and Koch *et al.*, 1991, *Science* 252:668-678). Several intracellular substrate proteins
that associate with receptor tyrosine kinases (RTKs) have been identified. They may
be divided into two principal groups: (1) substrates which have a catalytic domain;
and (2) substrates which lack such a domain but serve as adapters and associate with
catalytically active molecules (Songyang *et al.*, 1993, *Cell* 72:767-778). The
25 specificity of the interactions between receptors or proteins and SH2 domains of
their substrates is determined by the amino acid residues immediately surrounding
the phosphorylated tyrosine residue. Differences in the binding affinities between
SH2 domains and the amino acid sequences surrounding the phosphotyrosine
residues on particular receptors are consistent with the observed differences in their
30 substrate phosphorylation profiles (Songyang *et al.*, 1993, *Cell* 72:767-778). These
observations suggest that the function of each receptor tyrosine kinase is determined

not only by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signalling pathways recruited by specific growth factor receptors, as well as differentiation factor receptors.

Aberrant stimulation, expression or mutations in the PTKs have been shown to lead to either uncontrolled cell proliferation (*e.g.*, malignant tumor growth) or to defects in key developmental or reparative processes. Consequently, the biomedical community has expended significant resources to discover the specific biological role of members of the PTK family, their function in differentiation processes, their involvement in tumorigenesis and in other diseases, the biochemical mechanisms underlying their signal transduction pathways activated upon ligand stimulation and the development of novel drugs.

Tyrosine kinases can be of the receptor-type (having extracellular, transmembrane and intracellular domains) or the non-receptor type (being wholly intracellular).

Receptor Tyrosine Kinases (RTKs). The RTKs comprise a large family of transmembrane receptors with diverse biological activities. The receptor tyrosine kinase (RTK) family includes receptors that are crucial for the growth and differentiation of a variety of cell types (Yarden and Ullrich, *Ann. Rev. Biochem.* 57:433-478, 1988; Ullrich and Schlessinger, *Cell* 61:243-254, 1990). The intrinsic function of RTKs is activated upon ligand binding, which results in phosphorylation of the receptor and multiple cellular substrates, and subsequently in a variety of cellular responses (Ullrich & Schlessinger, 1990, *Cell* 61:203-212).

At present, at least nineteen (19) distinct RTK subfamilies have been identified. One RTK subfamily, designated the HER subfamily, is believed to be comprised of EGFR, HER2, HER3, and HER4. Ligands to the HER subfamily of receptors include epithelial growth factor (EGF), TGF- α , amphiregulin, HB-EGF, betacellulin and heregulin. Aberrant regulation of HER2/erbB2 kinase activity is believed to promote a transformed tumorigenic phenotype especially in breast carcinomas. Two other RTK subfamilies are designated the insulin receptor

subfamily, which is comprised of INS-R, IGF-1R and IR-R, and "PDGFR" subfamily which includes the PDGF α and β -receptors, CSF-1R, and c-kit.

Several receptor tyrosine kinases, and growth factors that bind thereto, have been suggested to play a role in angiogenesis, although some may promote angiogenesis indirectly (Mustonen and Alitalo, *J. Cell Biol.* 129:895-898, 1995). One such receptor tyrosine kinase, known as fetal liver kinase 1 (flk-1), is a member of the type III subclass of RTKs. An alternative designation for human flk-1 is kinase insert domain-containing receptor (KDR) (Terman et al., *Oncogene* 6:1677-83, 1991). Another alternative designation for flk-1/KDR is "vascular endothelial cell growth factor receptor 2" (VEGFR-2). The murine version of flk-1/VEGFR-2 has also been called NYK (Oelrichs et al., *Oncogene* 8 (1):11-15, 1993). DNAs encoding mouse, rat and human flk-1 have been isolated, and the nucleotide and encoded amino acid sequences reported (Matthews et al., *Proc. Natl. Acad. Sci. USA*, 88:9026-30, 1991; Terman et al., 1991, *supra*; Terman et al., *Biochem. Biophys. Res. Comm.* 187:1579-86, 1992; Sarzani et al., *supra*; and Millauer et al., *Cell* 72:835-846, 1993).

The type III subclass RTK designated *fms*-like tyrosine kinase-1 (flt-1) is related to flk-1/KDR (DeVries et al. *Science* 255:989-991, 1992; Shibuya et al., *Oncogene* 5:519-524, 1990). An alternative designation for flt-1 is "vascular endothelial cell growth factor receptor 1" (VEGFR-1). To date, members of the flk-1/KDR/VEGFR-2 and flt-1/ VEGFR-1 subfamilies have been found expressed primarily on endothelial cells. These subclass members are specifically stimulated by members of the vascular endothelial cell growth factor (VEGF) family of ligands (Klagsburn and D'Amore, *Cytokine & Growth Factor Reviews* 7: 259-270, 1996). Flt-1 is believed to be essential for endothelial organization during vascular development. Flt-1 expression is associated with early vascular development in mouse embryos, and with neovascularization during wound healing (Mustonen and Alitalo, *supra*). Expression of flt-1 in adult organs suggests an additional function for this receptor that is not related to cell growth (Mustonen and Alitalo, *supra*).

Another RTK that is related to flt-1 and flk-1/KDR is flt-4 (Galland et al., *Oncogene* 8:1233-40, 1993; Pajusola et al., *Oncogene* 8:2931-37, 1993). Features

shared by these three receptors include the seven immunoglobulin-like domains in their extracellular region. The amino acid sequence of flt-4 exhibits significant homology with the sequences of flt-1 and flk-1, especially in the tyrosine kinase domain (Galland et al., *supra*). Unlike flt-1 and flk-1/KDR, however, a precursor
5 form of flt-4 is cleaved during post-translational processing to form two disulfide-linked polypeptides (Pajusola et al., *supra*). Studies of flt-4 expression during development support the theory of venous origin of lymphatic vessels (Kaipainen et al., *Proc. Natl. Acad. Sci. USA* 92:3566-70, April, 1995).

Given the crucial role of endothelial cells in angiogenesis, growth factors that
10 act on endothelial cells are of particular interest for studies of the regulation of vascularization. One such factor is vascular endothelial cell growth factor (VEGF), which binds to both flk-1 and flt-1 with relatively high affinity and is mitogenic toward vascular endothelial cells (Terman et al., 1992, *supra*; Mustonen et al. *supra*; DeVries et al., *supra*). VEGF does not bind to flt-4 (Pajusola et al., *supra*). The
15 studies reported in Millauer et al., *supra*, suggest that VEGF and flk-1/KDR/VEGFR-2 are a ligand-receptor pair that play an important role in the formation and sprouting of blood vessels, termed vasculogenesis and angiogenesis, respectively.

Different forms of VEGF arising from alternative splicing of mRNA have
20 been reported, including the four species described by Ferrara et al. (*J. Cell. Biochem.* 47:211-218, 1991). Both secreted and predominantly cell-associated species of VEGF were identified by Ferrara et al. *supra*, and the protein is known to exist in the form of disulfide linked dimers.

It is also known that there are a variety of physiological and biochemical
25 mechanisms that underlie edema and the formation of the edematous state in an individual. An important mediator in one or more of these mechanisms is "vascular endothelial cell growth factor" (VEGF). This mediator is also known as "vascular permeability factor" (VPF). This factor upregulates transport in vascular endothelial cells, and causes an increase in the permeability of numerous vascular beds
30 including the skin, subcutaneous tissues, peritoneal wall, mesentery, diaphragm, trachea, bronchi, duodenum and uterus. Significant diapedesis, alterations in

exchange across the endothelium, extravasation and deposition of macromolecules at these sites and prolonged hypotension may accompany these increased permeability effects. These processes are thought to be a facilitating prelude to neovascularization. VEGF is expressed by inflammatory T-cells, macrophages, 5 neutrophils and eosinophils, etc., at sites of inflammation. This factor is upregulated by hypoxia, certain vasopressor hormones, growth factors, reproductive hormones and numerous inflammatory cytokines. VEGF-mediated vascular permeability has been implicated in such disorders as tumor ascites, endometriosis, edematous responses to burns and trauma, endothelial dysfunction in diabetes, ovarian 10 hyperstimulation syndrome complications, and ocular edema.

Thus, it is apparent that the inhibition of VEGF production or activity would be beneficial, especially to block the manifestation of the above-listed disorders. In particular, agents that are capable of blocking VEGF mediated hyperpermeability and edema and associated syndromes would be useful for alleviating these disorders.

15 Placenta growth factor (PlGF) has an amino acid sequence that exhibits significant homology to the VEGF sequence (Park et al., *J. Biol. Chem.* 269:25646-54, 1994; Maglione et al. *Oncogene* 8:925-31, 1993). As with VEGF, different species of PlGF arise from alternative splicing of mRNA, and the protein exists in dimeric form (Park et al., *supra*). PlGF binds flt-1 with high affinity, but not flk- 20 1/KDR (Park et al., *supra*). PlGF potentiates the mitogenic effect of VEGF on endothelial cells when VEGF is present at low concentrations, but has no detectable effect when VEGF is present at higher concentrations (Park et al., *supra*).

Two other subfamilies of RTKs have been designated as the FGF receptor family (FGFR1, FGFR2, FGFR3 and FGFR4) and the Met subfamily (c-met and 25 Ron).

Because of the similarities between the PDGF and FLK subfamilies, the two subfamilies are often considered together. The known RTK subfamilies are identified in Plowman *et al.*, 1994, *DN&P* 7(6):334-339, which is incorporated herein by reference.

30 Inhibiting angiogenesis is desirable in certain clinical situations (e.g., to suppress growth and metastasis of solid tumors, or in treating rheumatoid arthritis),

whereas promotion of vascularization is beneficial for treating other conditions (e.g., wound healing). Consequently, molecules that promote angiogenesis by transducing signals through the above-discussed receptors, and molecules capable of inhibiting such signal transduction, are both of interest.

5 *The Non-Receptor Tyrosine Kinases.* The non-receptor tyrosine kinases represent a collection of cellular enzymes which lack extracellular and transmembrane sequences. At present, over twenty-four individual non-receptor tyrosine kinases, comprising eleven (11) subfamilies (Src, Frk, Btk, Csk, Abl, Zap70, Fes/Fps, Fak, Jak, Ack and LIMK) have been identified. At present, the Src
10 subfamily of non-receptor tyrosine kinases is comprised of the largest number of PTKs and include Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk. The Src subfamily of enzymes has been linked to oncogenesis. A more detailed discussion of non-receptor tyrosine kinases is provided in Bolen, 1993, *Oncogene* 8:2025-2031, which is incorporated herein by reference.

15 Many of the tyrosine kinases, whether an RTK or non-receptor tyrosine kinase, have been found to be involved in cellular signaling pathways involved in numerous pathogenic conditions, including cancer, psoriasis, and other hyperproliferative disorders or hyper-immune responses.

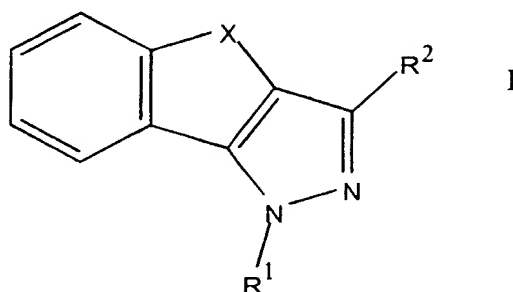
Development of Compounds to Modulate the PTKs. In view of the surmised
20 importance of PTKs to the control, regulation, and modulation of cell proliferation, the diseases and disorders associated with abnormal cell proliferation, many attempts have been made to identify receptor and non-receptor tyrosine kinase “inhibitors” using a variety of approaches, including the use of mutant ligands (U.S. Application No. 4,966,849), soluble receptors and antibodies (Application No. WO
25 94/10202; Kendall & Thomas, 1994, *Proc. Natl. Acad. Sci* 90:10705-09; Kim *et al.*, 1993, *Nature* 362:841-844), RNA ligands (Jellinek, *et al.*, *Biochemistry* 33:10450-56; Takano, *et al.*, 1993, *Mol. Bio. Cell* 4:358A; Kinsella, *et al.* 1992, *Exp. Cell Res.* 199:56-62; Wright, *et al.*, 1992, *J. Cellular Phys.* 152:448-57) and tyrosine kinase inhibitors (WO 94/03427; WO 92/21660; WO 91/15495; WO 94/14808; U.S. Patent
30 No. 5,330,992; Mariani, *et al.*, 1994, *Proc. Am. Assoc. Cancer Res.* 35:2268).

More recently, attempts have been made to identify small molecules which act as tyrosine kinase inhibitors. For example, bis monocyclic, bicyclic or heterocyclic aryl compounds (PCT WO 92/20642), and vinylene-azaindole derivatives (PCT WO 94/14808) have been described generally as tyrosine kinase inhibitors. Styryl compounds (U.S. Patent No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Patent No. 5,302,606), certain quinazoline derivatives (EP Application No. 0 566 266 A1), seleoindoles and selenides (PCT WO 94/03427), tricyclic polyhydroxylic compounds (PCT WO 92/21660) and benzylphosphonic acid compounds (PCT WO 91/15495) have been described as compounds for use as tyrosine kinase inhibitors for use in the treatment of cancer.

The identification of effective small compounds which specifically inhibit tyrosine signal transduction by modulating the activity of receptor and non-receptor tyrosine kinases to regulate and modulate abnormal or inappropriate cell proliferation is therefore desirable.

SUMMARY OF THE INVENTION

This invention is directed to compounds of the formula



wherein:

X is a carbonyl, a methylene or a substituted methylene group;

R¹ is a hydrogen or methyl group; and

R² is a pyridyl, a phenyl, or a substituted phenyl;

provided that when X is a methylene group, R¹ is a methyl group.

Enantiomers, tautomers, and mixtures of these compounds are included in this invention. Pharmaceutically acceptable acid addition salts of these compounds are also included in this invention.

The compounds of this invention are useful as inhibitors of the tyrosine kinase activity. In particular, the compounds of this invention are useful as inhibitors of tyrosine kinases that are important in the process of angiogenesis. These compounds are also useful as inhibitors of tyrosine kinases that are important in the process of vascular hyperpermeability. Since these compounds are anti-angiogenic and inhibit vascular hyperpermeability, they are important substances for inhibiting the progression disease states where angiogenesis and vascular hyperpermeability are important components.

Particularly preferred compounds of the present invention are compounds of formula I wherein R¹, R² and X shown in Table I:

TABLE I

R ¹	R ²	X
H	Phenyl	CO
H	4-methylphenyl	CO
H	4-fluorophenyl	CO
H	3-chlorophenyl	CO
H	4-chlorophenyl	CO
H	4-bromophenyl	CO
H	4-hydroxyphenyl	CO
H	3-methoxyphenyl	CO
H	4-methoxyphenyl	CO
H	4-hydroxy-3-methoxyphenyl	CO
H	3-pyridyl	CO
H	4-pyridyl	CO
H	3,4-(OCH ₃) ₂ phenyl	CO
H	phenyl	CH(OH)

CH ₃	phenyl	CO
CH ₃	4-methylphenyl	CO
CH ₃	3-chlorophenyl	CO
CH ₃	4-chlorophenyl	CO
CH ₃	4-bromophenyl	CO
CH ₃	4-hydroxyphenyl	CO
CH ₃	3-methoxyphenyl	CO
CH ₃	4-methoxyphenyl	CO
CH ₃	4-aminophenyl	CO
CH ₃	3-pyridyl	CO
CH ₃	4-chlorophenyl	CH ₂
CH ₃	4-methoxyphenyl	CH ₂
CH ₃	phenyl	CH(OH)
CH ₃	4-chlorophenyl	CH(OH)
CH ₃	3-pyridyl	CH(OH)
CH ₃	4-methoxyphenyl	CH(NH ₂)
CH ₃	phenyl	CH(NHC ₃ H ₇)
CH ₃	phenyl	CH(NHC ₂ H ₄ N(CH ₃) ₂)

The present invention further includes the use of these compounds in pharmaceutical compositions with a pharmaceutically effective amount of the above-described compounds and a pharmaceutically acceptable carrier or excipient. These pharmaceutical compositions can be administered to individuals to slow or halt the process of angiogenesis in angiogenesis-aided diseases.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention have antiangiogenic properties. For this reason, these compounds can be used as active agents against such disease states as arthritis, atherosclerosis, psoriasis, hemangiomas, myocardial angiogenesis, coronary and cerebral collateral vascularization, ischemic limb angiogenesis, wound healing, peptic ulcer *Helicobacter* related diseases, fractures, cat scratch fever,

rubeosis, corneal disease, neovascular glaucoma and retinopathies such as those associated with diabetic retinopathy or macular degeneration, or similar disease states dependent on angiogenesis-supported blood and nutrient supply or on endothelial cell hyperproliferative disorders. In addition, some of these compounds
5 can be used as antifertility agents or as abortifacients.

The compounds of this invention inhibit the catalytic activity of tyrosine kinases. That is, these compounds modulate signal transduction by tyrosine kinases. In particular, these compounds selectively inhibit the activity of the KDR/FLK-1/VEGFR-2 tyrosine kinases. Certain compounds of this invention also inhibit the
10 activity of additional tyrosine kinases such as flt-1/VEGFR-1, Src-subfamily kinases such as Lck, Src, fyn, yes, etc. The occurrence and potency of the inhibitory activity of the compounds of this invention against a particular tyrosine kinase is dependent on the nature, number and arrangement of the substituents (i.e. X, R¹ and R²) of these compounds.

The compounds of this invention, when administered to individuals in need
15 of such compounds, also inhibit vascular hyperpermeability and the formation of edema in these individuals. These compounds act, it is believed, by inhibiting the activity mediated by VEGF receptors which are involved in the process of vascular hyperpermeability and edema formation. VEGF is unique in that it is the only
20 angiogenic growth factor known to directly contribute to vascular hyperpermeability and the formation of edema. Indeed, vascular hyperpermeability and edema that is associated with the expression or administration of many other growth factors appears to be mediated via VEGF production. Diapedesis also often accompanies vascular hyperpermeability. Similarly, excessive vascular hyperpermeability can
25 disrupt normal molecular exchange across the endothelium in critical tissues and organs (e.g., lung and kidney), thereby perturbing function and causing macromolecular extravasation and deposition. By inhibiting the kinase activity of VEGF receptors, hyperpermeability, as well as associated extravasation, subsequent edema or ascites formation and matrix deposition is inhibited and minimized.

In addition, the N-methylated forms or N-methylated analogs of the compounds of this invention can also serve as prodrugs which act as metabolically activated antiangiogenic agents.

It is envisaged that the disorders listed above are mediated to a significant
5 extent by protein tyrosine kinase activity involving the KDR/VEGFR-2 and/or the flt-1/VEGFR-1 tyrosine kinases. By inhibiting the activity of these tyrosine kinases, the progression of the listed disorders or physiological conditions is inhibited because the angiogenic component of these disease states or physiological conditions is severely curtailed. The action of the compounds of this invention, by
10 their selectivity for specific tyrosine kinases, result in a minimization of side effects that would occur if less selective tyrosine kinase inhibitors were used.

The potency and specificity of the generic compounds of this invention can often be altered and optimized by substituent variations and conformational restrictions.

15 In this invention, the following definitions are applicable:

“Pharmaceutically acceptable acid addition salt” refers to those salts which retain the biological effectiveness and properties of the free bases and which are obtained by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid or with organic acids such as sulfonic
20 acid, carboxylic acid, organic phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, lactic acid, tartaric acid and the like.

In the compounds of this invention, the substituents of R^2 , when R^2 is a phenyl, can be a lower alkyl of 1 or 2 carbon atoms, a halogen atom, a lower alkoxy group, a hydroxyl group or an amino group. In the compounds of this invention, X
25 can be a carbonyl group, a methylene group, a hydroxymethylene group, an amino methylene group or a lower alkyl amino methylene group.

Pharmaceutical Formulations and Routes of Administration

The identified compounds of this invention can be administered to a human
30 patient by themselves or in pharmaceutical compositions where they are mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders.

A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms. Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest
5 edition.

Routes of Administration

Suitable routes of administration may, for example, include oral, eyedrop, rectal, transmucosal, topical, or intestinal administration; parenteral delivery,
10 including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the compound in a local rather than a systemic manner, for example, via injection of the compound intra-articularly (e.g.,
15 for rheumatoid arthritis), often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Composition/Formulation

20

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

25 Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration
30 chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation.

5 Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, 10 suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by combining the active compound with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as 15 sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, 20 agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. 25 Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active 30 ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft

capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

- 5 For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable
10 propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound
15 and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g. bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions,
20 solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions.
25 Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which
30 increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases
5 such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly or by intramuscular injection). Thus, for example, the compounds may be formulated with
10 suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-
15 miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system
20 dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the
25 fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples
30 of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater

toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may,
5 depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are
10 not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the PTK modulating compounds of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric,
15 sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protic solvents than are the corresponding free base forms.

Effective Dosage

Pharmaceutical compositions suitable for use in the present invention include
20 compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed
25 disclosure provided herein.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cellular assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cellular assays (i.e., the concentration of the test
30 compound which achieves a half-maximal inhibition of the PTK activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD₅₀ and ED₅₀. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the kinase modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data; e.g. the concentration necessary to achieve 50-90% inhibition of the kinase using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using the MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

5 Packaging

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

- 10 Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of a cellular hyperproliferative disorder, inhibition of angiogenesis, treatment of fibrosis, diabetes, edema, ascites and the like.

15

EXEMPLIFICATIONS

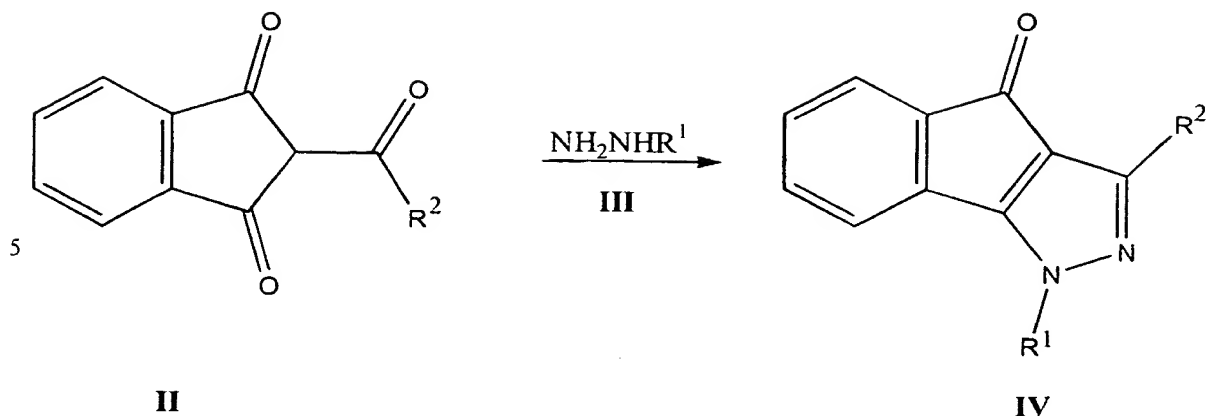
I. Synthesis of Compounds

The compounds of this invention, formula I, can be synthesized by using the following scheme.

20

Synthesis of indeno[1,2-c]pyrazole ring system:

- The indeno[1,2-c]pyrazole ring system can be synthesized by the methods of Braun and Mosher (Braun, R. A.; Mosher, W. A. *J. Am. Chem. Soc.* 1958, 80, 4919 and Braun, R. A.; Mosher, W. A. *J. Org. Chem.* 1959, 24, 648) by reacting the
- 25 appropriately acylated 1,3-indandione with hydrazine or methylhydrazine.



10 Functionalization of the bridging carbon:

The bridging carbonyl can be transformed to a methylene group via a Wolf-Kishner reduction of the corresponding hydrazone (Mosher, W.A., Tawfik, E.-Z., Lipp, D. W. *J. Org. Chem.* 1971, 36, 3890).

Additional methods for functionalization of the bridging carbonyl and
 15 specific examples can be found in Japanese Patent Application JP 60 130521 A2, and B. Loev, U.S. Patent 3,004,983 (1960).

Examples

The invention is further illustrated by the following Examples which are given by way of example only. The final product of each of these Examples was
 20 characterized by one or more of the following procedures: high performance liquid chromatography; elemental analysis, nuclear magnetic resonance spectroscopy, infrared spectroscopy and high resolution mass spectroscopy. The following abbreviations are used:

DMF = dimethylformamide;
 25 IMS = industrial methylated spirits; and
 LCMS = liquid chromatography/mass spectroscopy

Examples 1 and 2 were prepared by methods outlined in JP60-130521.

Example 1

3-(3,4-Dimethoxyphenyl)indeno[1,2-c]pyrazol-4(1*H*)-one, (commercially available from Menai Organics Ltd., Unit 5, Menai Technology Centre Deiniol Road, Bangor Gwynedd, N. Wales LL57 2UP UK.)

5

Example 2

3-(3,4,5-Trimethoxyphenyl)indeno[1,2-c]pyrazol-4(1*H*)-one, m.p.268-272°C.

Example 3

- 10 a) Dimethyl phthalate (15.0 g) in tetrahydrofuran (100 ml) was added to a stirred suspension of sodium methoxide (8.31 g) in tetrahydrofuran (60 ml) followed by 4'-chloroacetophenone (11.8 g) in tetrahydrofuran (100 ml) at 20°C under nitrogen. The mixture was then boiled under reflux for 18 hours then cooled to 20°C and poured into ether (250 ml) and water (250 ml). The aqueous layer was separated
15 and the organic layer was washed with water (3 x 75 ml). The combined aqueous extract and washings were acidified with concentrated hydrochloric acid and the solid which precipitated was collected by filtration. The filtrate was extracted with dichloromethane, dried and evaporated. The residue was recrystallized from ethanol to give 2-(4-chlorobenzoyl)-indan-1,3-dione.

20

- b) The product from a) (1.23 g) was suspended in ethanol (40 ml) and hydrazine hydrate (0.216 g) was added under nitrogen. The mixture was boiled under reflux under nitrogen for 4 hours, then allowed to cool to ambient temperature and left standing for 16 hours, then filtered to give 3-(4-chlorophenyl)indeno[1,2-c]pyrazol-
25 4(1*H*)-one, m.p. >330°C.

Example 4

- 3-(4-Chlorophenyl)indeno[1,2-c]pyrazol-4(1*H*)-one (0.68 g) was dissolved in N,N-dimethylformamide (30 ml) under nitrogen at 20°C. Sodium hydride (106 mg, 30
30 of a 60% dispersion in mineral oil) was added and the mixture was stirred for 30 minutes, then iodomethane (0.38 g) was added. The mixture was stirred at 20°C

for 2 hours and then poured into water (50 ml). A yellow solid was collected by filtration and washed with petroleum ether (25 ml). The solid was dissolved in hot ethyl acetate and pre-absorbed onto silica which was applied to the top of the silica column. The mixture was separated by flash column chromatography using
5 dichloromethane/petroleum ether/methanol (50:50:1). Fractions were collected, combined as appropriate, and evaporated to give a solid which was crystallised from ethyl acetate to give 3-(4-chlorophenyl)-1-methylindeno[1,2-c]pyrazol-4(1*H*)-one, m.p. 219-221°C. $\delta_{\text{H}}[^2\text{H}_6]$ DMSO (250MHz) 4.07 (3H, s, NMe), 7.41 (1H, t, Ar), 7.55 (4H, m, Ar), 7.65 (1H, d, Ar-8-H), 8.13 (2H, d, Ar). The structure was
10 identified by the presence of a Nuclear Overhauser Effect.

Example 5

a) In a similar manner to Example 4, 3-phenylindeno[1,2-c]pyrazol-4(1*H*)-one (1.0 g) was reacted with iodomethane (0.64 g) at 20°C to give, after separation by
15 flash column chromatography, 1-methyl-3-phenylindeno[1,2-c]pyrazol-4(2*H*)-one, m.p. 187-188°C.

Example 6

A mixture of 3-(3,4-dimethoxyphenyl)indeno[1,2-c]pyrazol-4(1*H*)-one (0.6
20 g) and sodium borohydride (0.16 g) in absolute ethanol (100 ml) was boiled under reflux for 5 hours. The reaction mixture was cooled and the ethanol removed under reduced pressure. Water (20 ml) was added and the mixture was extracted with ethyl acetate (3 x 50 ml). The combined ethyl acetate extracts were washed with water, dried, filtered and evaporated to give a solid which was purified by
25 preparative high performance liquid chromatography on a Dynamax C18 reverse phase (41.4 x 250 mm) column using acetonitrile/triethylammonium formate buffer as eluant to give 3-(3,4-dimethoxyphenyl)-1,4-dihydroindeno[1,2-c]pyrazol-4-ol, m.p. 197-199°C.

Example 7

A mixture of 3-phenylindeno[1,2-c]pyrazol-4(1*H*)-one (0.86 g), sodium borohydride (0.26 g) and absolute ethanol (200 ml) was stirred at ambient temperature for 72 hours. The mixture was filtered to remove a small amount of solid which was discarded and the filtrate was concentrated under reduced pressure to low volume whereupon crystals were formed. This suspension was diluted with water to approximately 250 ml and the solid was collected by filtration and recrystallized from methanol to give 3-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-4-ol, m.p. 269-272°C.

Example 8

a) A mixture of indane-1,3-dione (5.85 g), piperidine (0.46 ml) and pyridine-4-carboxaldehyde (4.31 g) was stirred in IMS (40 ml) at ambient temperature for 50 minutes. The mixture was cooled in an ice-bath but no crystallization occurred. The solution was allowed to warm to ambient temperature and then the solvent was removed under reduced pressure to give a residue which was partially purified by flash column chromatography on silica using 10% industrial methylated spirit in dichloromethane followed by 20% industrial methylated spirit in dichloromethane as the mobile phases. The leading fractions were collected, combined and evaporated to give a solid which was triturated with ice-cold diethyl ether and filtered to give 2-(4-pyridylmethylene) indan-1,3-dione, m.p. 152-157°C.

b) The product from a) (0.64 g) was dissolved in a mixture of dichloromethane (5.5 ml) and methanol (5.5 ml) and the mixture was stirred at ambient temperature as 2M aqueous sodium hydroxide solution (1.36 ml) was added, followed by 100 volume aqueous hydrogen peroxide (0.55 ml). The mixture was stirred at ambient temperature for 45 minutes. Water (25 ml) and dichloromethane (25 ml) were added and the aqueous phase was separated and extracted with dichloromethane. The combined dichloromethane extracts were evaporated under reduced pressure to give 1,3-dioxo-3'-(4-pyridyl)-spiro[indan-2,2'-oxirane] in a crude form which was used directly in the next part of this example.

c) A mixture of the epoxide from b) (0.51 g), methanol (20 ml), glacial acetic acid (20 drops) and hydrazine hydrate (1 ml of a solution prepared by diluting hydrazine hydrate 1 ml to 10 ml total volume with methanol) was boiled under reflux for 18 hours. The mixture was allowed to cool and then the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica using 5% and then 10% IMS in dichloromethane as the mobile phase. Appropriate fractions were combined and evaporated to give a solid which was triturated with diethyl ether and filtered to give 3-(4-pyridyl)indeno[1,2-c]pyrazol-4-(1*H*)-one, m.p. >300°C.

Example 9

a) A 1:1 mixture (by volume) of dichloromethane:methanol (1 ml) was added to 2-(4-fluorobenzylidene)-1,3-indandione (50.7 mg) in a septum sealed tube added via a Gilson 215 liquid handler, followed by aqueous 2M sodium hydroxide solution (100.5 µl) followed by 100 volumes aqueous hydrogen peroxide (41.1 µl). The resulting reaction mixture was then shaken at ambient temperature for 20 hours. Following LCMS analysis, further 100 volumes aqueous hydrogen peroxide (41.1 µl) was added, this time manually via a plastic-tipped pipette. Shaking was then continued for a further 24 hours.

The reaction mixture was equilibrated between dichloromethane (approx. 5 ml) and water (approx. 2 ml), filtering off the organic phase through an Empore® filter cartridge and washing through with dichloromethane (approx. 2 ml). The dichloromethane phase was evaporated and the residue further dried in vacuo to give 46 mg of material. The product was taken on to the next step on the basis of being the corresponding 2,3-epoxyketone.

b) n-Butanol (2 ml) was added via a Gilson 215 liquid handler to the product from a) in a septum sealed tube, followed by hydrazine hydrate as a 10% solution by

volume in n-butanol (100 μ l, 1.2 molar equivalents). Finally, glacial acetic acid (2 drops) was added manually by syringe. The reaction mixture was heated at 100° C for 6 hours. The reaction mixture was evaporated to dryness and the residue was purified by chromatography on silica eluting with ethyl acetate, diluted as necessary with petroleum ether b.p. 40-60°C. Appropriate fractions were evaporated and dried in vacuo. The product was further purified by preparative HPLC to give 3-(4-fluorophenyl)indeno[1,2-c]pyrazol-4(1*H*)-one (1.3 mg, retention time 4.42 minutes and with MH⁺ corresponding to C₁₆H₉FN₂O=264 by LCMS). The identity of the products was confirmed by LCMS using the conditions given below.

10

LC

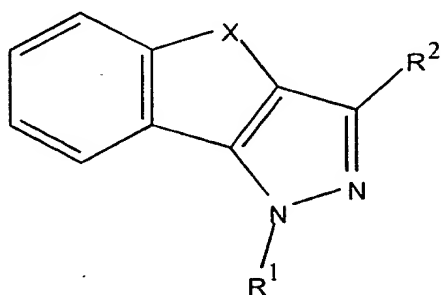
Column:	5 μ HYPERSIL BDS C18 (100 X 2.1 mm)
Mobile Phase:	0.1% Formic Acid : MeCN (gradient – see below)
Conditions:	10-100% MeCN in 8 minutes
15 (gradient)	100% MeCN for 1 minute
	100-10% MeCN in 2 minutes
	(Total analysis run time 11 minutes)
Flow Rate:	1 ml/min
Wavelength Range:	206-320 nm
20 Injection Volume:	10 μ l

MS

Ionization	APCI +ve/-ve
Mass Range:	150-500 m/z
25 Cone Voltage:	20

The chemical structures of the compounds produced in these Examples are shown in Table II:

TABLE II



Ex No.	X	R ¹	R ²
1	CO	H	3,4-(OCH ₃) ₂ phenyl
2	CO	H	3,4,5-(OCH ₃) ₃ phenyl
3	CO	H	4-Cl phenyl
4	CO	CH ₃	4-Cl phenyl
5	CO	CH ₃	Phenyl
6	CHOH	H	3,4-(OCH ₃) ₂ phenyl
7	CHOH	H	Phenyl
8	CO	H	4-pyridyl
9	CO	H	4-F phenyl

II. *In Vitro* PTK Assays

The following *in vitro* assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the PTKs. Similar assays can be designed along the same lines for other tyrosine

kinases using techniques well known in the art.

A. KDR Protein Production Using Baculovirus System:

The coding sequence for the KDR intra-cellular domain (aa789-1354) was generated through PCR using cDNAs isolated from HUVEC cells. A poly-His₆ sequence was introduced at the N-terminus of this protein as well. This fragment was cloned into transfection vector pVL1393 at the Xba 1 and Not 1 site.

Recombinant baculovirus (BV) was generated through co-transfection using the BaculoGold Transfection reagent (PharMingen). Recombinant BV was plaque purified and verified through Western analysis. For protein production, SF-9 cells were grown in SF-900-II medium at 2×10^6 /ml, and were infected at 0.5 plaque forming units per cell (MOI). Cells were harvested at 48 hours post infection.

B. Purification of KDR

SF-9 cells expressing (His)₆KDR(aa789-1354) were lysed by adding 50 ml of Triton X-100 lysis buffer (20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1mM PMSF, 10µg/ml aprotinin, 1 µg/ml leupeptin) to the cell pellet from 1L of cell culture. The lysate was centrifuged at 19,000 rpm in a Sorval SS-34 rotor for 30 min at 4°C. The cell lysate was applied to a 5 ml NiCl₂ chelating sepharose column, equilibrated with 50 mM HEPES, pH7.5, 0.3 M NaCl. KDR was eluted using the same buffer containing 0.25 M imidazole. Column fractions were analyzed using SDS-PAGE and an ELISA assay (below) which measures kinase activity. The purified KDR was exchanged into 25mM HEPES, pH7.5, 25mM NaCl, 5 mM DTT buffer and stored at -80°C.

C. Human Tie-2 Kinase Production and Purification

The coding sequence for the human Tie-2 intra-cellular domain (aa775-1124) was generated through PCR using cDNAs isolated from human placenta as a template. A poly-His₆ sequence was introduced at the N-terminus and this construct was cloned into transfection vector pVL 1939 at the Xba 1 and Not 1 site. Recombinant BV was generated through co-transfection using the BaculoGold Transfection reagent (PharMingen). Recombinant BV was plaque purified and verified through Western analysis. For protein production, SF-9 insect cells were grown in SF-900-II medium at 2×10^6 /ml, and were infected at MOI of 0.5. Purification of the His-tagged kinase used in screening was analogous to that described for KDR.

D. Human Flt-1 Tyrosine Kinase Production and Purification

The baculoviral expression vector pVL1393 (Phar Mingen, Los Angeles, CA) was used. The nucleotide sequence encoding the kinase domain was generated through PCR using cDNA libraries isolated from HUVEC cells. A nucleotide
5 sequence encoding poly-His₆ was placed 5' to the nucleotide region encoding the entire intracellular kinase domain of human Flt-1 (amino acids 786-1338). The histidine residues enabled affinity purification of the protein as a manner analogous to that for KDR (Part B.) and ZAP70 (Part F.) SF-9 insect cells were infected at a 0.5 multiplicity and harvested 48 hours post infection.

10

E. Lck and EGFR Tyrosine Kinase Sources

Lck or truncated forms of Lck were commercially obtained (e.g. Upstate Biotechnology Inc., Saranac Lake, NY or Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or were purified from known natural or recombinant sources using
15 conventional methods. EGFR was purchased from Sigma (Cat # E-3641; 500 units/50 µl) and the EGF ligand was acquired from Oncogene Research Products/Calbiochem (Cat # PF011-100).

F. ZAP70 Tyrosine Kinase Production

20 The Baculoviral expression vector pVL 1393 (Phar Mingen, Los Angeles, CA) was used. A nucleotide sequence encoding poly-His₆ was placed 5' to the nucleotide region encoding the entire ZAP70 (amino acids 1-619). The nucleotide sequence encoding the ZAP70 coding region was generated through PCR using cDNA libraries isolated from Jurkat immortalized T-cells. The histidine residues
25 enabled affinity purification of the protein. The LVPRGS bridge constituted a recognition sequence for proteolytic cleavage by thrombin, thereby enabling removal of the affinity tag from the enzyme. SF-9 insect cells were infected at a 0.5 multiplicity and harvested 48 hours post infection.

G. Purification of ZAP70

SF-9 cells were lysed in a buffer containing 20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM PMSF, 1 µg/ml leupeptin, 10 µg/ml aprotinin and 1 mM sodium orthovanadate. The soluble lysate was applied to a
5 chelating Sepharose Hi Trap column (Pharmacia) equilibrated in 50 mM HEPES, pH 7.5, 0.3 M NaCl. The fusion protein was eluted with 250 mM imidazole. The recovered enzyme was stored in buffer containing 50 mM HEPES, pH 7.5, 50 mM NaCl and 5 mM DTT.

H. Enzyme Linked Immunosorbent Assay (ELISA)

10 Enzyme linked immunosorbent assays (ELISA) can be used to detect and measure the presence of tyrosine kinase activity. The ELISA can be conducted according to known protocols which are described in, for example, Voller, *et al.*, 1980, "Enzyme-Linked Immunosorbent Assay," In: *Manual of Clinical Immunology*, 2d ed., edited by Rose and Friedman, pp 359-371 Am. Soc. of
15 Microbiology, Washington, D.C.

The disclosed protocol can be adapted for determining activity with respect to a specific RTK. For example, preferred protocols for conducting the ELISA experiments for KDR is provided below. Adaptation of these protocols for determining a compound's activity for other members of the RTK family, as well as
20 non-receptor tyrosine kinases, are well within the abilities of those in the art. For purposes of determining inhibitor selectivity, a universal PTK substrate (e.g., random copolymer of poly (Glu,Tyr) 20,000-50,000 MW) was employed together with ATP (typically 5µM) at concentrations approximately twice the apparent K_m in the assay.

25

KDR *IN VITRO* ELISA

The following procedure was used to assay the inhibitory effect of compounds of this invention on KDR tyrosine kinase activity:

30 Buffers and Solutions:

1. PGT: Poly (Glu,Tyr) 4:1

Store powder at -20°C. Dissolve powder in phosphate buffered saline (PBS) for 50mg/ml solution. Store 1ml aliquots at -20°C. When making plates dilute to 250µg/ml in Gibco PBS.

5 2. Reaction Buffer:

100mM Hepes, 20mM MgCl₂, 4mM MnCl₂, 5mM DTT, 0.02%BSA,
200µM NaVO₄, ph 7.10

3. ATP: Store aliquots of 100mM at -20°C. Dilute to 20µM in water.

10

4. Washing Buffer:

PBS with 0.1% Tween 20

5. Antibody Diluting Buffer:

15 0.1% bovine serum albumin (BSA) in PBS

6. TMB Substrate:

Mix TMB substrate and Peroxide solutions 9:1 just before use or use K-Blue
Substrate from Neogen

20

7. Stop Solution:

1M Phosphoric Acid

Procedure

25 1. Plate Preparation:

Dilute PGT stock (50mg/ml, frozen) in PBS to a 250µg/ml. Add 125µl per
well of Corning modified flat bottom high affinity ELISA plates (Corning #25805-
96). Add 125µl PBS to blank wells. Cover with sealing tape and incubate overnight
37°C. Wash 1x with 250µl washing buffer and dry for about 2hrs in 37°C dry

30 incubator.

Store coated plates in sealed bag at 4°C until used.

2. Tyrosine Kinase Reaction:

-Prepare inhibitor solutions at a 4x concentration in 20% DMSO in water.

-Prepare reaction buffer

5 -Prepare enzyme solution so that desired units are in 50 μ l, e.g. for KDR
make to 1 ng/ μ l for a total of 50ng per well in the reactions. Store on ice.

-Make 4x ATP solution to 20 μ M from 100mM stock in water. Store on ice

-Add 50 μ l of the enzyme solution per well

-Add 25 μ l 4x inhibitor

10 -Add 25 μ l 4x ATP for inhibitor assay

-Incubate for 10 minutes at room temperature

-Stop reaction by adding 50 μ l 0.05N HCl per well

-Wash plate

**Final Concentrations for Reaction:

15 ATP: 5 μ M

5% DMSO

3. Antibody Binding

-Dilute 1mg/ml aliquot of PY20-HRP (Pierce) antibody to 50ng/ml in 0.1%

20 BSA in PBS by a 2 step dilution (100x, then 200x)

-Add 100 μ l Ab per well. Incubate 1 hr at room temp. Incubate 1 hr at 4C.

-Wash 4x plate

4. Color reaction

25 -Prepare TMB substrate and add 100 μ l per well

-Monitor OD at 650nm until 0.6 is reached

-Stop with 1M Phosphoric acid. Shake on plate reader.

-Read OD immediately at 450nm

Optimal incubation times and enzyme reaction conditions vary slightly with
30 enzyme preparations and are determined empirically for each lot.

Analogous assay conditions were used for Flt-1, Tie-2, EGFR and ZAP70. For Lck, the Reaction Buffer utilized was 100 mM MOPSO, pH 6.5, 4 mM MnCl_2 , 20 mM MgCl_2 , 5 mM DTT, 0.2% BSA, 200 mM NaVO_4 under the analogous assay conditions.

5

PKC kinase source

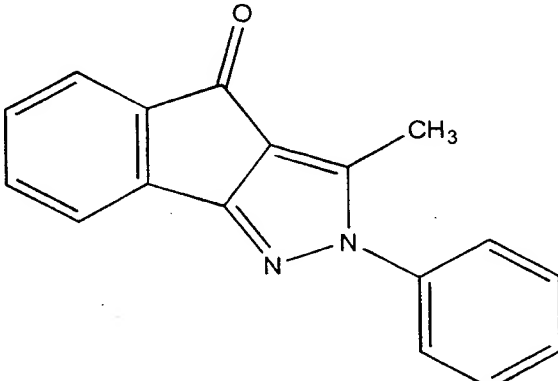
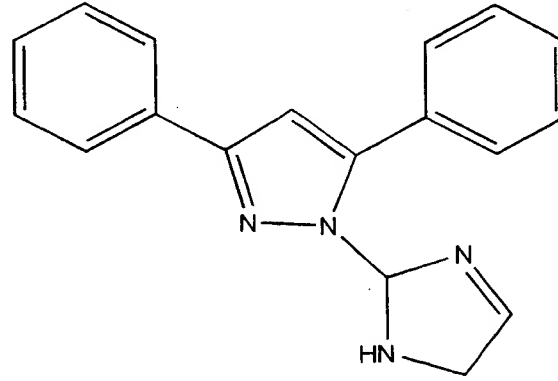
The catalytic subunit of PKC may be obtained commercially (Calbiochem).

PKC kinase assay

10 A radioactive kinase assay was employed following a published procedure (Yasuda, I., Kirshimoto, A., Tanaka, S., Tominaga, M., Sakurai, A., Nishizuka, Y. *Biochemical and Biophysical Research Communication* 3:166, 1220-1227 (1990)). Briefly, all reactions were performed in a kinase buffer consisting of 50 mM Tris-HCl pH7.5, 10mM MgCl_2 , 2mM DTT, 1mM EGTA, 100 μM ATP, 8 μM peptide,
15 5% DMSO and ^{33}P ATP (8Ci/mM). Compound and enzyme were mixed in the reaction vessel and the reaction initiated by addition of the ATP and substrate mixture. Following termination of the reaction by the addition of 10 μL stop buffer (5 mM ATP in 75mM phosphoric acid), a portion of the mixture was spotted on phosphocellulose filters. The spotted samples were washed 3 times in 75 mM
20 phosphoric acid at room temperature for 5 to 15 minutes. Incorporation of radiolabel was quantified by liquid scintillation counting.

Results

The following inhibitory concentrations for representative compounds were obtained:

5	Compound of Formula I where:	KDR ELISA IC ₅₀
	X is carbonyl, R ¹ is hydrogen and R ² is phenyl	0.15 μM
10	X is carbonyl, R ¹ is hydrogen and R ² is 4-methylphenyl	2.6 μM
	X is carbonyl, R ¹ is hydrogen and R ² is 4-methoxyphenyl	1.5 μM
15		>100 μM
20		
25		>100 μM
30		

These results demonstrate that compounds of the present invention have notable inhibitory activity for KDR tyrosine kinase, particularly when compared to the lack of inhibitory activity for KDR tyrosine kinase by compounds outside the scope of the present invention.

5

III. Cellular RTK Assays

The following cellular assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the RTKs. Similar assays can be designed along the same lines for other tyrosine kinases using techniques well known in the art.

10

A. VEGF-Induced KDR Phosphorylation in Human Umbilical Vein Cells (HUVEC) as Measured by Western Blots.

1. HUVEC cells (from pooled donors) were purchased from Clonetics (San Diego, CA) and cultured according to the manufacturer directions. Only early passages (3-8) were used for this assay. Cells were cultured in 100 mm dishes (Falcon for tissue culture; Becton Dickinson; Plymouth, England) using complete EBM media (Clonetics).

20

2. For evaluating a compound's inhibitory activity, cells were trypsinized and seeded at $0.5-1.0 \times 10^5$ cells/well in each well of 6-well cluster plates (Costar; Cambridge, MA).

3. 3-4 days after seeding, plates were 90-100% confluent. Medium was removed from all the wells, cells were rinsed with 5-10ml of PBS and incubated 18-24h with 5ml of EBM base media with no supplements added (i.e., serum starvation).

25

4. Serial dilutions of inhibitors were added in 1ml of EBM media (25uM, 5uM, or 1uM final concentration) to cells and incubated for one hour at 37°

30

C. Human recombinant VEGF₍₁₆₅₎ (R&D Systems) was then added to all the wells in 2 ml of EBM medium at a final concentration of 50ng/ml and incubated at 37° C for 10 minutes. Control cells untreated or treated with VEGF only were used to assess background phosphorylation and induction by VEGF.

5

5. All wells were then rinsed with 5-10ml of cold PBS containing 1mM Sodium Orthovanadate (Sigma) and cells were lysed and scraped in 200µl of RIPA buffer (50mM Tris-HCl pH7, 150mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1mM EDTA) containing protease inhibitors (PMSF 1mM, Aprotinin 1µg/ml, 10 Pepstatin 1µg/ml, Leupeptin 1µg/ml, Na Vanadate 1mM, Na Fluoride 1mM) and 1µg/ml of DNase (all chemicals from Sigma Chemical Company, St. Louis, MO). The lysate was spun at 14,000 rpm for 30min, to eliminate nuclei.

6. Equal amounts of proteins were then precipitated by addition of cold 15 (-20 C) Ethanol (2 volumes) for a minimum of 1 hour or a maximum of overnight. Pellets were reconstituted in Laemli sample buffer containing 5% β-mercaptoethanol (BioRad; Hercules, CA) and boiled for 5min. The proteins were resolved by polyacrylamide gel electrophoresis (6%, 1.5mm Novex, San Deigo, CA) and transferred onto a nitrocellulose membrane using the Novex system. After blocking 20 with bovine serum albumin (3%), the proteins were probed overnight with anti-KDR polyclonal antibody (C20, Santa Cruz Biotechnology, Santa Cruz, CA) or with anti-phosphotyrosine monoclonal antibody (4G10, Upstate Biotechnology, Lake Placid, NY) at 4°C. After washing and incubating for 1 hour with HRP-conjugated F(ab)₂ of goat anti-rabbit or goat-anti-mouse IgG the bands were visualized using the emission 25 chemiluminescence (ECL) system (Amersham Life Sciences, Arlington Height, IL).

For example, when X is carbonyl, R¹ is hydrogen and R² is phenyl in a compound of Formula I, the cellular IC₅₀ according to this method is 3 µM.

B. HUVEC Mitogenesis Assay

30 Human umbilical vein endothelial cells (HUVEC) were cultured in EGM-Complete Media (endothelial growth media supplemented with 10% FBS,

hydrocortisone, epidermal growth factor and bovine brain extract as per the supplier's directions). Cells, media and supplements were purchased from Clonetics (San Diego, CA). The HUVEC Mitogenesis Assay was used to measure inhibition of mitogenesis induced by VEGF, FGF or Complete Media. Cells were harvested by
5 trypsinization, washed once and suspended in Complete Media (CM) at a concentration of 5,000 cells/0.15 ml. Cells were seeded into 96-well plates at 5,000 cells/well and incubated at 37° C for 24 hours. Cells were washed once with serum-free media (SFM) and incubated under serum-free conditions for 24 hours. Non-serum starved control wells were cultured with CM during this period. Test
10 compounds were prepared as 10 mM stock solutions in DMSO, diluted in SFM and added to wells over a concentration range (0.005-50 μ M final concentration). Cells were incubated for 1 hour prior to addition of 50 ng/ml VEGF or FGF (R & D Systems, Minneapolis, MN). Complete media stimulated wells received the test compound and stimulus simultaneously. After 5 hours of incubation, 0.5 Ci/well of
15 tritiated thymidine (Amersham) was added and the cells were incubated overnight. The assay was stopped by freezing plates at -20° C for 24 hours. Plates were harvested onto filter mats with a Tomtec Harvester and counted with a Betaplate liquid scintillation counter (Wallac).

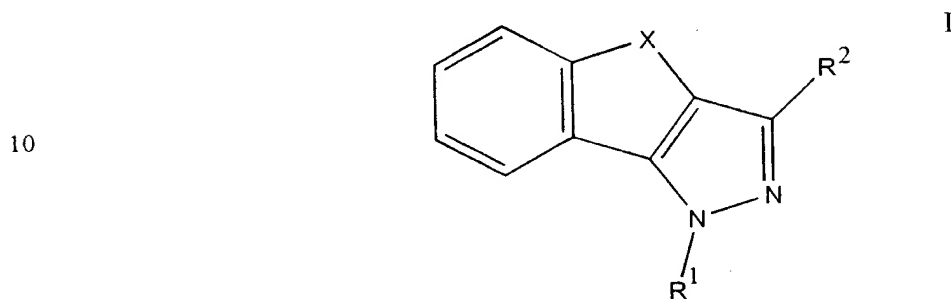
For example, when X is carbonyl, R¹ is hydrogen and R² is phenyl in a
20 compound of Formula I, the HUVEC mitogenesis IC₅₀ according to this method is <0.10 μ M.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in
25 the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be
30 encompassed in the scope of the claims.

CLAIMS

What is claimed is:

- 5 1. A method of inhibiting tyrosine kinase activity comprising the administration of a compound represented by the formula:



15 wherein:

X is a carbonyl, a methylene or a substituted methylene group;

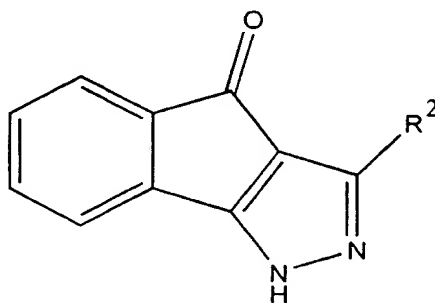
R¹ is a hydrogen or methyl group; and

20 R² is a pyridyl, a phenyl, or a substituted phenyl group to said tyrosine kinase in sufficient concentration to inhibit the enzyme activity of said tyrosine kinase; provided that when X is a methylene group, R¹ is a methyl group.

25 2. The method of Claim 1 wherein said compound is in an enantiomeric form, a tautomeric form, is in a mixture with one or more other said compounds, or is both in an enantiomeric form and in a mixture with one or more other said compounds.

30 3. The method of Claim 1 wherein said tyrosine kinase is either a receptor tyrosine kinase or a non-receptor tyrosine kinase.

4. The method of Claim 3 wherein said tyrosine kinase is selected from the group consisting of KDR, flt-1, TIE-2, Lck, Src, fyn, and yes.
5. The method of Claim 1 wherein the activity of said tyrosine kinase affects angiogenesis.
6. The method of Claim 5 wherein the inhibition of said tyrosine kinase is anti-angiogenic.
7. The method of Claim 6 wherein said inhibition of said tyrosine kinase inhibits the progression of the disease state selected from the group consisting of arthritis, atherosclerosis, psoriasis, hemangioma, myocardial angiogenesis, coronary and cerebral collateral vascularization, ischemic limb angiogenesis, corneal disease, rubeosis, neovascular glaucoma, macular degeneration, wound healing peptic ulcer *Helicobacter* related diseases, fractures, diabetic retinopathy and cat scratch fever.
8. The method of Claim 1 wherein the activity of said tyrosine kinase affects vascular hyperpermeability.
9. The method of Claim 1 wherein said compound is represented by the formula:



wherein R₂ is selected from the group consisting of phenyl, 4-methylphenyl, 4-fluorophenyl, 3-chlorophenyl, 4-chlorophenyl, 4-bromophenyl, 4-hydroxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 3-methoxy-4-hydroxyphenyl, 3-pyridyl, 4-pyridyl and 3,4-dimethoxyphenyl.

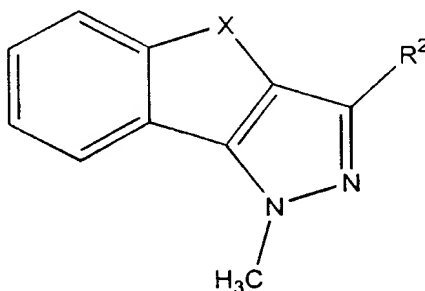
5

10. The method of Claim 9 wherein R₂ is selected from the group consisting of phenyl, 4-fluorophenyl and 4-hydroxyphenyl.

10

11. The method of Claim 1 wherein said compound is represented by the formula:

15



20

wherein R₂ and X are together respectively selected from the group consisting of phenyl, CO; 4-methylphenyl, CO; 3-chlorophenyl, CO; 4-chlorophenyl, CO; 4-bromophenyl, CO; 4-hydroxyphenyl, CO; 3-methoxyphenyl, CO; 4-methoxyphenyl, CO; 4-aminophenyl, CO; 3-pyridyl, CO; 4-chlorophenyl, CH₂; 4-methoxyphenyl, CH₂; phenyl, CH(OH); 4-chlorophenyl, CH(OH); 3-pyridyl, CH(OH); 4-methoxyphenyl, CH(NH₂); phenyl, (CH(NHC₃H₇)); and phenyl, CH(NHC₂H₄N(CH₃)₂).

25

12. The method of Claim 11 wherein X is a carbonyl group and R₂ is selected from the group consisting of 4-chlorophenyl, 4-bromophenyl, 4-hydroxyphenyl, 3-methoxyphenyl and 4-methoxyphenyl.

13. The method of Claim 1 wherein R¹ is a methyl group, wherein the inhibition of said tyrosine activity is associated with anti-fertility or abortifacient effects.
- 5 14. The method of Claim 1 wherein X is a carbonyl or a substituted methylene group and R¹ is a hydrogen, wherein the inhibition of said tyrosine activity is associated with anti-fertility or abortifacient effects.

INTERNATIONAL SEARCH REPORT

Inter. Appl. No.

PCT/US 98/21259

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/415

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3 932 430 A (HABECK DIETMAR A ET AL) 13 January 1976 see column 11, line 22 - line 23 see column 11, line 26 ---	1-4, 13
X	PATENT ABSTRACTS OF JAPAN vol. 009, no. 287 (C-314), 14 November 1985 & JP 60 130521 A (MORISHITA SEIYAKU KK), 12 July 1985 cited in the application see abstract see figure 1 ---	1-6, 9-11
A	US 5 593 997 A (DOW ROBERT L ET AL) 14 January 1997 see claim 1 see abstract -----	1-14



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

24 November 1998

Date of mailing of the international search report

03/12/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Trifilieff-Riolo, S

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/21259

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-14
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/21259

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3932430 A	13-01-1900	AU 4765672 A	26-04-1974
		BE 797964 A	09-10-1973
		BE 806671 A	29-04-1974
		BE 789948 A	11-04-1973
		DE 2317716 A	02-05-1974
		DE 2249644 A	19-04-1973
		FR 2157852 A	08-06-1973
		NL 7213549 A	17-04-1973
		NL 7304722 A	02-05-1974
		DD 100256 A	12-09-1973
		JP 48044254 A	26-06-1973
		ZA 7207315 A	28-08-1974
		DD 105225 A	12-04-1974
<hr/>			
US 5593997 A	14-01-1997	NONE	
<hr/>			